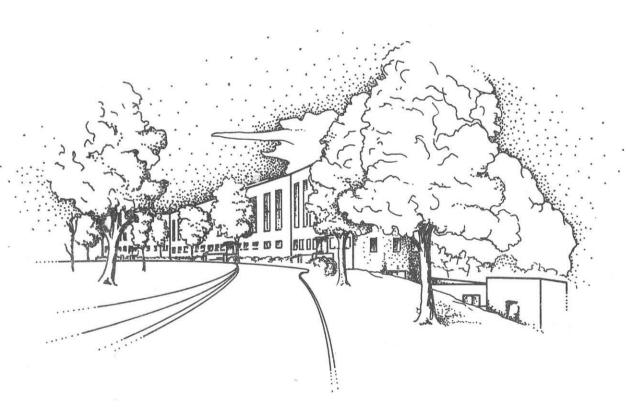


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Differential Behavioral Effects of Breathing Air and Helium-Oxygen At Three to Ten Atmospheres

John R. Thomas and Arthur J. Bachrach

Project MF12.524.004.7007D

Research Report No. 2

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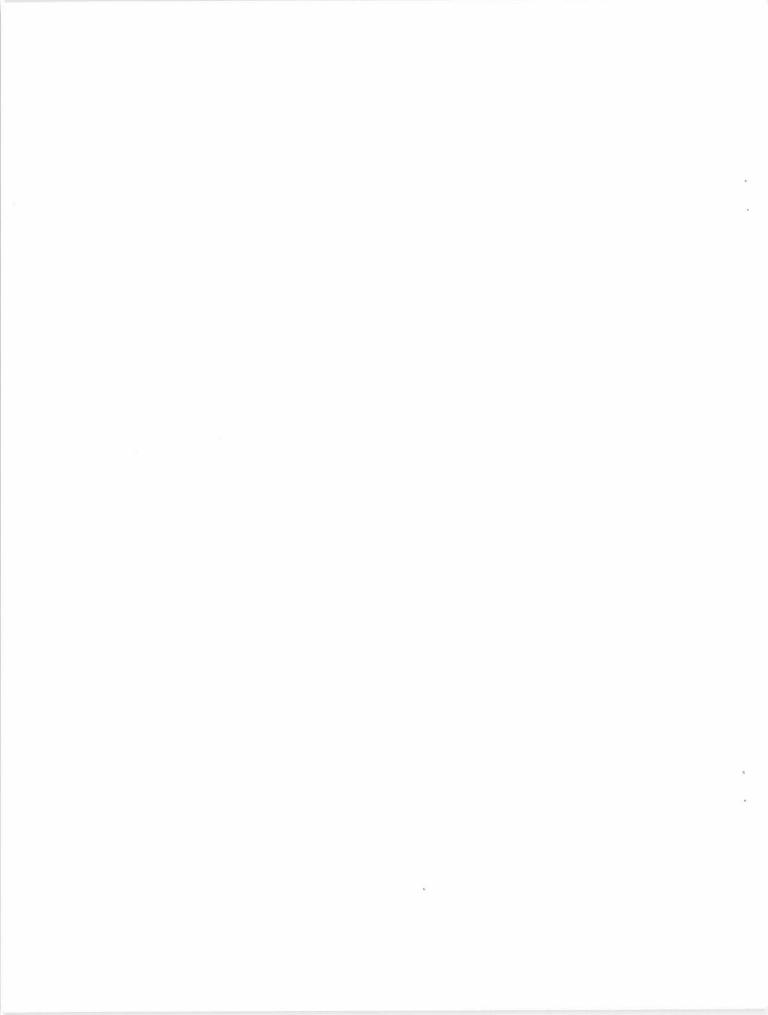
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#### ABSTRACT

A multiple schedule of reinforcement, consisting of a fixed-ratio (FR) schedule and a fixed-interval (FI) schedule, was used as an ongoing behavioral baseline to measure differential gas and depth effects on pigeons. Measurements were taken at depths of 66, 99, 200, and 300 feet. An 80% helium-20% oxygen mixture was found to have less disruptive effects than air on pigeons responding on the two different schedules particularly at 200- and 300-foot depths. Repeated exposure to pressure, independent of gas mixture or depth, resulted in a gradual behavioral adaptation measured by a reduction in the disruption of behavior.

### KEY WORDS

Hyperbaric animal research
Operant conditioning
Hyperbaric pressure
Hyperbaric performance
Multiple schedules
Helium
Compressed air
Adaptation

## DIFFERENTIAL BEHAVIORAL EFFECTS OF BREATHING AIR AND HELIUM-OXYGEN AT THREE TO TEN ATMOSPHERES

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The inert gas helium is usually substituted for that part of air made up of nitrogen as one way to reduce the effects of inert gas narcosis, or "nitrogen narcosis," at depths below 100 to 200 feet. This gas substitution has been employed for over 30 years, since the early work done by Behnke and Yarbrough, and End. 12 The use of this gas substitution has increased greatly since development of the techniques of saturation diving. 10,13

A number of studies, using a wide variety of measurements, have shown that a helium-oxygen (heliox) gas mixture is less narcotic than air at depth. 1,2,4,6,7,8,9 In these studies, measurement of narcotic effects has usually been indicated by reporting a smaller decrement or change in the obtained performance. However, the variables of which the performance is a function, including the tasks themselves, have differed from study to study making precise quantitative comparisons difficult. Previous research 10,16,17 has indicated the development of a

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This experiment was conducted according to the principles set forth in the "Guide for Laboratory Animal Facilities and Care" prepared by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences, National Research Council.

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behavioral technology which allows for quantifiable, controlled and highly predictable patterns of behavior in hyperbaric conditions. The techniques permit a degree of experimental control never before attained, which makes possible the systematic study of behavioral phenomena in individual organisms, and allows for direct functional comparisons between experiments.

In this experiment the investigators extended the behavioral technology techniques to more complex baselines than previously to compare the effects of a heliox mixture to air under several different simulated depths. The range of species investigated in hyperbaric environments was also extended to White Carneaux pigeons, an organism used extensively in the experimental analysis of behavior and in behavioral pharmacology.

#### METHOD

#### Subjects

The subjects were two adult White Carneaux pigeons weighing 560 grams (Subject P-W) and 525 grams (Subject P-R) when allowed free access to grain and water. The subjects were maintained at about 80% of their free-feeding weight during the study.

#### Apparatus

The experimental cage in which the pigeons performed was similar in design to that described by Ferster and Skinner. 13 The cage contained a single plastic response key which the subjects were trained to peck for grain reinforcements. The response key was mounted behind a hole in the front wall, and could be illuminated by either red or green colored lights located behind it. The cage was illuminated during an experimental session

by a single houselight located on the top of the front wall. Directly below the response key an opening in the wall allowed access to a grain magazine which could be raised into place to provide access to the grain reinforcer. The key light and houselight went off simultaneously with the operation and illumination of the grain magazine.

During training of the subjects and many of the baseline control sessions, the experimental cage was mounted inside a sound-attenuated enclosure with a filtered ventilating fan.

Automatic programming of the experiments was accomplished by a system of solid state digital logic equipment connected by cable to the cage. Data were recorded on electromechanical counters and a cumulative recorder.

The experimental pressure sessions (simulated dive sessions), noise-control sessions, and most baseline control sessions were carried out with the cage mounted inside a steel hyperbaric chamber. The chamber is cylindrical in shape, can withstand internal pressure of 1,000 psi, and is provided with threaded openings to permit connections to the gas supply and the programming equipment. Gas mixtures used were either compressed air or 80% helium-20% oxygen (heliox).

#### Procedure

Sessions were run daily and terminated after 60 4-second presentations of mixed grain reinforcements, or about 2 hours, whichever occurred first. Each session was preceded and followed by a blackout condition, variable in duration, during which all lights in the experimental cage were off and responses had no programmed consequences.

The two pigeons were trained to perform on a complex reinforcement schedule, technically known as a multiple fixed-ratio, fixed-interval schedule. This schedule involves two different reinforcement schedules that alternate unsystematically with each other. A distinctive stimulus is associated with each schedule.

When the plastic response key was illuminated with red light a fixedratio (FR) schedule of 30 responses was in effect. Under this FR schedule
the subject was required to emit 30 pecks on the response key to produce
4 seconds of access to grain. Such a schedule usually produces, after
an initial brief pause, a high and rapid rate of responding until grain
reinforcement occurs. When the response key was illuminated with green
light, the alternative schedule was in effect. This schedule was a fixedinterval (FI) schedule of 3 minutes; the first key-peck to occur after
3 minutes elapsed produced 4 seconds of access to grain. Responses before
the 3-minute interval elapsed had no effect, and were merely recorded.
An FI schedule usually produces a low rate of responding early in the
interval with response rate increasing in a positive acceleration as the
interval passes.

The entirely different patterns of responding on the FR and FI schedules were brought under the discriminative stimulus control of the two colored lights, so that either of the two types of behavior could be produced "on demand" by the presentation of the appropriate stimulus light. The two schedules alternated in an unsystematic fashion, with the change, if it occurred, following the presentation of a grain reinforcement.

The same schedule could occur two or three times in succession, but usually alternated after each reinforcement. The arrangement was such that each schedule appeared for approximately half of an experimental session. Time, and the number of responses, were recorded separately for the FR and FI schedules so that response rates (responses/min) could be calculated for both schedules.

After stable daily response rates were obtained on the multiple schedule, the subjects were exposed to a number of auditory control sessions in the hyperbaric chamber. Compressed air was allowed to flow into the chamber with all valves open so that ambient pressure was maintained; however, the subjects were exposed to the noise associated with gas flow.

The subjects were then exposed to sessions with either compressed air or 80% helium-20% oxygen at hyperbaric pressures equivalent to 66, 99, 200, and 300 feet of sea water. The actual exposure order to the depths and two different gas mixtures is presented in Table 1 (p. 6). All dive sessions were separated from each other by at least 7 to 10 days with control baseline sessions in between. The only exception to this was at the 300-foot depth where the four exposures to 300 feet for the two gas mixtures occurred on successive days.

TABLE 1
Order of Exposure to Depths and Gases

Subject P-W			Subject P-R			
Depth	Gas		Depth	Gas		
99	HeO <sub>2</sub>		66	HeO <sub>2</sub>	- 2	
66	Air		66	Air		
66	HeO <sub>2</sub>		99	HeO2		
99	Air		99	Air		
200	HeO <sub>2</sub>		200	HeO <sub>2</sub>		
200	Air	***	200	Air		
300	HeO <sub>2</sub>		300	HeO <sub>2</sub>		
300	Air		300	Air		
300	HeO <sub>2</sub>		300	HeO2		
300	Air		300	Air		

The duration of each experimental dive varied between 1 1/2 and 2 hours, depending on the depth. Compression rate was approximately 8 feet per minute (ft/min) for all depths. Decompression rate was approximately 7 psi per minute (psi/min) with three-minute stops at 80, 59, 34, and 6 psi as appropriate, depending on the depth. Time at depth was one hour.

#### RESULTS

Baseline performance on the multiple FR-FI schedule may be seen in Figures 1A and 2A for the two subjects. The multiple schedule controlled two different patterns of responding. When the red key light was on and the FR schedule was in effect, responding usually began immediately and continued at a high and steady rate until reinforcement occurred. Subject P-W responded on the FR schedule at an average rate of 280 responses/min. Subject P-R responded on the FR schedule at an average rate of 192 responses/min. When the green key light was on and the FR schedule was in effect, responding was absent or low for a period of time; it began to increase as the three-minute interval passed, reaching the highest rate just before the availability of reinforcement. The average rate of responding on the FI schedule was 16 responses/min for P-W and 35 responses/min for P-R.

Presented in Figures 3 and 4 are the changes in performance rate on the multiple schedule under compressed air and heliox during the one-hour bottom time at 66, 99, 200 and 300-foot depths. The data

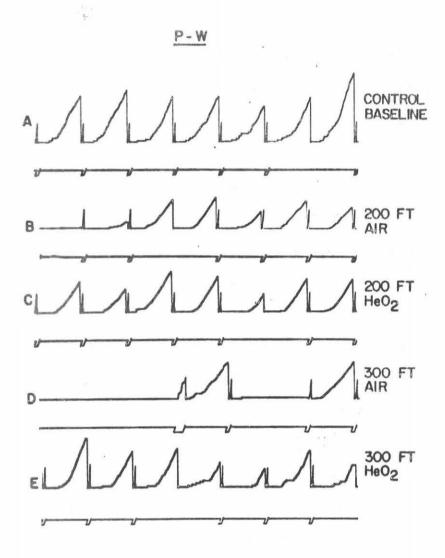


Figure 1. Segments of cumulative response records for Subject P-W from Control (A) and air and heliox dive sessions.

Excursions of the recording pen during which the bottom pen is up indicate FI schedules; excursions with the bottom pen down are FR schedules. The recording pen resets to baseline each time reinforcement occurs.

[HeO2: 80% helium-20% oxygen (heliox)]

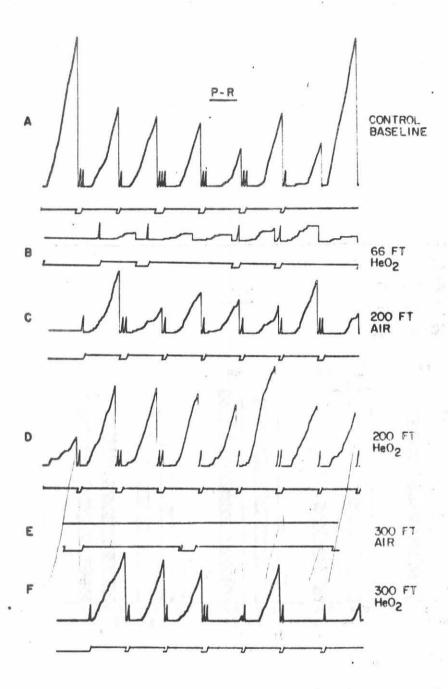


Figure 2. Segments of cumulative response records for Subject P-R from Control (A) and air and heliox dive sessions.

Excursions of the recording pen during which the bottom pen is up indicate FI schedules; excursions with the bottom pen down are FR schedules. The recording pen resets to baseline each time reinforcement occurs.

[HeO2: 80% helium-20% oxygen (heliox)]

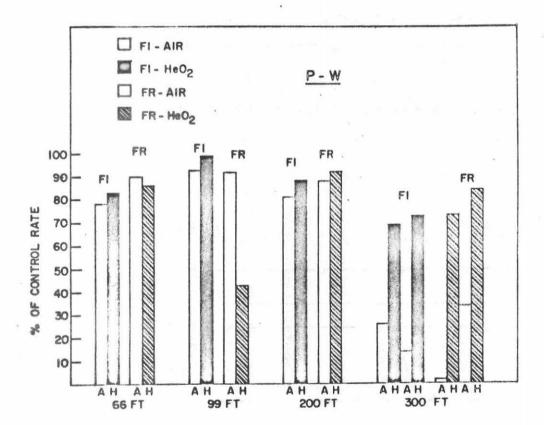


Figure 3. Percent of control response rates on the multiple FI-FR schedule under air and heliox at 66, 99, 200, and 300-foot depths for Subject P-W.

[NeO2: 80% helium-20% oxygen (heliox)]

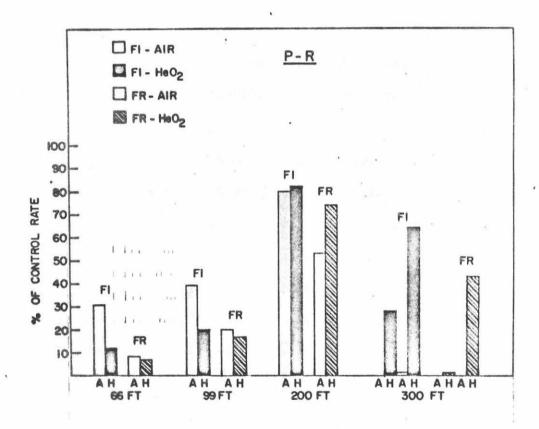


Figure 4. Percent of control response rates on the multiple FI-FR schedule under air and heliox at 66, 99, 200, and 300-foot depths for Subject P-R.

[HeO2: 80% helium-20% oxygen (heliox)]

are presented in terms of percent change in response rate from that of control baseline rates. The figures show that for both gas mixtures at all depths there was a decrease in response rates below that of control rates. Two general main results emerged as a function of exposure to the hyperbaric conditions: (a) there was a general behavioral adaptation in terms of magnitude of effect with each subsequent exposure to hyperbaric pressure; and (b) the more important result was the differential gas effects on response rates at the deeper depths.

The subjects' performance rates at 200-foot depth on both FR and FI schedules decreased more under compressed air than under the heliox mixture. The data presented in Figures 3 and 4 are percent changes in response rates, thus the absolute changes in FR rates are much greater than those of FI rates due to the difference in their initial control rate values. The gas-depth effects are differentially affected by the nature of the schedule of reinforcement; in this case, FR rates declined more. Segments of cumulative records for bottom time under the two gas mixtures at 200-foot depth are shown in Figures 1B, 1C, 2C, and 2D.

Clearer differential effects between the two gas mixtures are apparent at 300-foot depth where air and heliox pressure dives were alternated on a daily basis. Response rates for both subjects on both FR and FI schedules were lower under compressed air than under this heliox mixture. Almost complete cessation of responding occurred for subject P-R at 300-foot depth under air. Portions of cumulative response records during bottom time at 300-foot depth for the two gas mixtures may be seen in Figures 1D, 1E, 2E, and 2F.

Differential gas and behavioral effects are less clear for the 66-foot and 99-foot dives. This may have been due to adaptation effects to hyperbaric pressure and could have been a function of the sequential order of exposure to the depths and gases. The initial hyperbaric exposure for P-W was 99 feet under heliox. The largest decrement in FR rate of any depth for either gas is seen for this dive. The adaptation effects are perhaps more apparent for Subject P-R, whose first dive was at 66 feet under heliox. A segment of the cumulative record from this dive is shown in Figure 2B. Performance actually increased across gases and depths for this subject, with better performance at 200 feet for both gas mixtures than at a depth of 66 feet. The general adaptation process is not so apparent at the shallower depths for Subject P-W (with the exception of heliox at 99 feet).

At 200 and 300 feet, response rates were higher under heliox than under air even though exposure to heliox always occurred first (Table 1). Nevertheless, rates were higher under the second exposure to heliox than the first for both subjects on both schedules, which suggests that adaptation may still interact with behavior at the deeper depths. At the deeper depths, (particularly 300 feet) appropriate behavior was better maintained with heliox than with compressed air.

#### DISCUSSION

One of the conclusions of this experiment is that heliox at 200- and 300-foot depths is less disruptive to ongoing operant behavior than is

compressed air, although heliox displays some disruptive effects. The superiority of heliox over air has been demonstrated in a number of different hyperbaric situations. 1,2,4,6,7,8,9 The experiment also demonstrates the less disruptive effects of heliox with pigeons responding on a response key on two different schedules of reinforcement, and extends the general findings to another species, never before observed under hyperbaric conditions. It provides for the measurement of narcotic effects on an exact. behaviorally-controlled baseline which permits precise quantification. A decline in response rates on FR and FI schedules as a function of gas mixture and depth may be one of the better measures offered for the quantification of inert gas effects. Previous research from this laboratory has demonstrated the usefulness of behavioral response-rate changes in the assessment of inert gas narcosis. 16,17 The results of this study also confirm conclusions of earlier research in other laboratories 5,6,15 that repeated exposure to pressure per se, independent of gas or depth, apparently results in a gradual adaptation by the organism as measured by a gradual reduction in the disruption of behavior.

#### APPENDIX

#### Specifications of Apparatus and Chamber

The basic operant apparatus used in the experiment is a Harvard Instrument Company cage (8 1/2 inches wide, 9 1/2 inches long, and 10 inches high) made with aluminum front and back panels and perforated plexiglass sides and top. The floor of the cage is comprised of wire mesh. Two Lehigh Valley Electronics pigeon response keys are mounted behind two 1/4-inch diameter holes on the front panel. The response keys are located 7 3/4 inches from the floor and are 3 1/2 inches center to center apart. A 2 x 2 1/2inch opening in the front panel, centered 3 inches above the floor, gives access to grain reinforcements which are presented by a Lehigh Valley Electronics grain feeder located behind the panel. The response keys can be transilluminated by any number of colors or geometrical figures contained in one-plane digital display units (Industrial Electronic Engineers, Inc.) located behind each of the two keys. Centered 3 1/2 inches from the top on the front panel is a 28 VDC houselight with a yellow lens cover. The entire apparatus (cage, grain feeder) is mounted on a sheet metal base with all electrical connections to the apparatus terminating on a blue ribbon connector mounted on the end of the sheet metal base.

During training and most baseline sessions, the apparatus was mounted on steel slides inside a BRS-Foringer cage housing which is a sound-reducing airtight enclosure, 18 1/2 inches high x 29 inches wide x 16 inches deep, with a filtered ventilating fan.

All of the pressure runs and some noise control sessions were conducted with the basic apparatus mounted on a set of slides inside a Bethlehem hyperbaric chamber. The chamber is cylindrical with internal dimensions of 42 inches in length and 18 inches in diameter. The chamber can withstand internal pressures of 1,000 pounds per square inch (psi) which is comparable to a depth of 2,245 feet of sea water. The chamber is penetrated with several threaded openings for pressure-fitted connectors to the gas supply and the various programming instrumentation associated with the test apparatus. Across the upper inside surface of the chamber is a metal plate with heating and cooling coils which are thermostatically controlled to maintain constant temperature (24-26°C).

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